INTRODUCTION:

Every year 500 million people infected with malaria and 2.5 million people died of it throughout the world (Nussenzweig R. S. and Zavala F., 1997). Malaria has been a major parasitic, communicable tropical disease transmitted by the biting of female *Anopheles* mosquito and caused by four *Plasmodium* species namely *Plasmodium vivax, P. falciparum, P. ovale* and *P. malariae.* Among them *P. vivax* and *P. falciparum* are common in India. The reemergence of malaria has been reported in several countries such as India, Peru, China and Korea (Sharma P., 1996). It has become a serious health problem in these countries. In India during year 2011 total malaria cases reported were 336,545. Among them 53.75% were due to *Plasmodium falciparum* and rest of the cases were due to *P. vivax* (http://www.flutrackers.com/forum/showthread.php?t=170884). In India, nine *Anopheline* vectors are involved in transmitting malaria in diverse geo-ecological paradigms. About 2 million confirmed malaria cases and 1,000 deaths are reported annually, although 15 million cases and 20,000 deaths are estimated by WHO South East Asia Regional Office. India contributes 77% of the total malaria in Southeast Asia (Kumar A. *et al.*, 2007 & Chadee D. D., Maitre A. L. and Tillukdharry C. C., 1992). Valsad district of Gujarat state, India, is considered as one of the malaria endemic area (Srivastava H. C. and Yadav R. S., 2000). Delay in malaria treatment may lead to serious consequences including death. Prompt and effective treatment is also important for controlling the transmission of malaria.

Fever is the cardinal symptom of malaria. It can be intermittent with or without periodicity or continuous. Many cases have chills and rigors. The fever is often accompanied by headache, myalgia, arthralgia, anorexia, nausea and vomiting. The symptoms of malaria can be non-specific and mimic other diseases like viral infections, enteric fever etc. (Guidelines for treatment and diagnosis of malaria in India, 2011). In these circumstances it is not just possible to diagnose the malaria clinically. Microscopic examination of blood smears stained with Romanosky's stain is the most common technique to diagnose malaria since last hundred years (Rougemont M *et al.*, 2004). Microscopy continues to be the gold standard for identification of *Plasmodium* species in the laboratories (Makler M. T., Palmer C. J. and Ager A. L., 1998). The method is easy to apply and cost effective in the laboratories where skilled professionals are available who can even detect very low level of parasite like 10 to 50 parasites/µl. So the sensitivity of microscopy may fluctuate depending upon the skill of technician. There are many other techniques like Polymerase Chain Reaction, Quantitave Buffy coat–acridine orange staining, Enzyme Linked Immunosorbent Assay and Rapid Diagnostic Tests available for malaria diagnosis. Among them WHO has recognized RDT as simple and cost effective diagnostic test for malaria to overcome the deficiencies of microscopy and clinical diagnosis (Basco L. K. *et al.*, 1995). RDT detects *P. falciparum* Histidine-Rich-Protein II (HRP II) antigen and parasite Lactate Dehydrogenase (pLDH) antigen present in all four *Plasmodium* species (Noedl H. *et al.*, 2002). It does not require any special equipment and give results within 15 to 30 minutes (Singh N., Valecha M. and Sharma V. P., 1997).

There are several different approaches to the assessment of parasite's susceptibility to antimalarial drugs such as chloroquine, artemisinin derivatives, coartem, atovaquone, amodiaquine hydrochloride etc. (White N. J., 2002). WHO has recommended several *in vivo* methods (WHO, 1973). The genetic diversity of parasites has been exploited to develop

molecular protocols whereby true recrudescence could be distinguished from re-infections. An *in vivo* method involves administration of drug to the patient. Blood is collected before and after treatment and is tested to see any decrease in parasitemia. One such guideline suggested by WHO is to collect blood before treatment and after 48 hours of treatment. It should lead to decrease in parasite count by less than 25%. Parasite count greater than 25% suggest high degree resistance, inappropriate therapy or inadequate drug absorption (Hoffman S. L., 1992). Besides *in vitro* tests are rapid and precise, they may have several disadvantages like drugs acting through active metabolite cannot be studied, non producibility of pharmacokinetic effects, toxic compounds also get selected, expertise and infrastructure needed, lack of clinical correlation etc. (Kalra B. S. *et al.*, 2006) Modes of action of some antimalarial drugs are listed below (http://www.malariasite.com):

Antimalarial Drug	**Mode of Action**
Arteether	Schizonticidal, binds heme iron and generate oxygen radicals.
Artemether	Same as above
Artesunate	Same as above
Chloroquine	Schizonticidal, preventing the polymerization of heme to hemezoin in food vacuole.
Sulfadoxine + Pyrimethamine	Schizonticidal, Gametocytidal effect, Inhibit DNA synthesis
Primaquine	Active against hepatic stages, mode of action not known.
Amodiaquine hydrochloride	Schizonticidal, preventing the polymerization of heme to hemezoin in food vacuole.
Lumefantrine	Schizonticidal, act on food vacuole, inhibit protein & nucleic acid synthesis.

Bioinformatics now a day become a valuable field to solve many biological problems and even for malaria. BLAST, Clustal W, T Coffee, Phylodendron are few important online soft wares, Swissmodel, Swissdock helpful in *in silico* study. Drug resistance malaria parasites may emerge through mutations in the active sites or from biochemical changes in the drug receptors. The continued search for new molecular drug targets for drug design may widen the therapeutic strategies to fight drug resistance in human malaria (Penna-Coutinho J. et al., 2011). We can perform docking studies using structures of components of medicinal plants, i. e. tulsi - *Ocimum sanctum* and mamejavo - *Enicostemma littorale*. *Enicostemma littorale,* a herb commonly used as a bitter tonic also called as chhota chirayta. It is also reported to possess antitumor, hypoglycemic and antimalarial activities (Katewa S. S. and Arora A., 2001). Tulsi have been widely studied for curing various ailments due to its great therapeutic potential. Number of pharmacologic effect such as antimicrobial, hypoglycemic, immunomodulatory, antistress, CNS depressant, anti ulcerogenic, antitumor etc. have been observed by various workers (Das S. K. and Vasudevan D. M., 2006). Antimalarial properties of these plant constituents can be checked

and proved against various enzymes/proteins of parasite in dry lab work to minimize the wet lab work.

LITERATURE REVIEW:

- Howard et al. in 1986 and Makler & Hinrichs in 1993 showed presence of Histidine-rich protein II and parasite lactate dehydrogenase in Plasmodium falciparum and other non-falciparum species respectively. These two antigens proved highly valuable in development of RDTs (Howard R. J. *et al.*, 1986 & Makler M. T. and Hinrichs D. J., 1993).
- Iqbal J. et al. in 1999 compared Microscopy, PCR and RDT OptiMAL for malaria diagnosis and found no reliability of OptiMAL RDT (Iqbal J. *et al.*, 1999).
- Marie-pierre Brenier-Pinchart et al. in 2000 compared Parasight-F along with microscopy and QBC and found difficulty of interpreting the results of the Parasight-F test in discordance with microscopy (Pinchart M. P. B. *et al.*, 2000).
- Hwan Sub Lim et al. in 2001 used two RDTs and ELISA with microscopy and suggested RDT as a good alternative for conventional microscopy (Lim H. S. and Kim H. S., 2001).
- Kumar K. R. et al. in 2004 tested efficacy of Paracheck-Pf for diagnosis of P. falciparum malaria and reported high sensitivity and specificity of this test (Kumar K. R. and Sudarshan K. S., 2004).
- Rodulfo H. et al. in 2007 evaluated RDT OptiMAL along with PCR and microscopy. They found high reliability of both RDT and PCR over to use of microscopy (Rodulfo H. *et al.*, 2007).
- Ahmed S. E. et al. in 2007 tested efficacy of Paracheck-Pf with microscopy and showed high concordance of both test with each other (Ahmed S. E. *et al.*, 2007).
- The development of drug resistance against quinine, chloroquine, sulfa drugs, mefloquine atovaquone and artemisinin were first reported respectively in the year 1910, 1957, 1967, 1982, 1996 and 2001 (http://www.malariasite.com).
- In 1973 WHO gave guidelines regarding in vivo and in vitro antimalarial drug susceptibility testing (WHO, 1973).
- Stepniewska K. et al. in 2004 tested in vivo drug efficacy against Plasmodium falciparum. They collected blood samples before treatment and after 2, 7, 14, 28 and 42 of treatment to see reduction/reoccurrence of parasitemia (Stepniewska K. *et al.*, 2004).
- Sisowath C. in 2009 conducted drug efficacy study and collected blood samples on days 1, 2, 3, 7, 14, 28, 35 and 42 (Sisowath C., 2009).
- Assefa A. et al. in 2010 conducted study to check efficacy of coartem. The follow ups of patients were taken on days 1, 2, 3, 7, 14, 21 and 28 from finger prick samples and proved coartem as excellent antimalarial drug (Assefa A. *et al.*, 2010).
- Berwal R. et al. in 2006 amplified successfully both full and partial Plasmodium vivax LDH gene from Indian strains (Berwal R. *et al*., 2006).
- Chaikuad A. et al. in 2005 solved crystal structure of Plasmodium vivax LDH (Chaikuad A. *et al.*, 2005).
- Wiwanitkit V. in 2007 compared Plasmodium and human LDH and found that though the sequences of both LDH are quite different but the molecular functions are similar, LDH cannot selectively used as drug target protein (Wiwanitkit V., 2007).

- Singh N. et al. in 2009 performed in silico studies on enzymes histone acetyltransferase and sarcoendoplasmic reticulum Calcium ATPase and found that curcumin, artemisinin and diarylheptanoids may result in potent antimalarial drug (Singh N. and Misra K., 2009).
- Prakash N. et al. in 2010 performed docking study on enzyme dihydrofolate reductase against drug proguanil and modified drug compounds using software Arguslab and Hex and found modified compounds much better than commercial drugs (Prakash N. *et al*., 2010).
- Coutinho J. P. et al. in 2011 performed docking studies on Plasmodium falciparum LDH against some chemical compounds and conclude that in silico approach is less expensive for discovering new antimalarial drugs and LDH is a good drug target protein (Penna-Coutinho J. *et al*., 2011).

JUSTIFICATION AND LIKELY BENEFITS:

The popular malaria diagnosis method microscopy involves good time and tough microscopic observation. Skilled professional is required to observe the same. As an option RDTs are widely available but still it is a question mark to replace microscopy with RDT. It is easy to perform that even a new lab technician or a layman can also perform it and interpret the results. Even the RDT is able to differentiate between *P. falciparum* and *P. vivax*.

Confirmed *P. vivax* cases are treated with chloroquine in full therapeutic dose for three days. To prevent relapse primaquine is given daily for 14 days under supervision. Recently *Plasmodium falciparum* malaria is treated with artemisinin based combination therapy, quinoline compounds, amodiaquine, mefloquine etc. In *Plasmodium falciparum* malaria Artemisinin Combination Therapy is given to all confirmed *P. falciparum* cases found positive by microscopy or RDT. This is to be accompanied by single dose primaquine on Day 2 (http://www.mrcindia.org/Guidelines%20for%20Diagnosis2011.pdf). Despite the arsenal of drugs available for treatment of malaria, it remains a worldwide public health problem. *P. falciparum* quickly develop resistance under selective drug pressure. Hence we require an array of new drugs for malaria treatment. Lactate dehydrogenase is an enzyme that catalyses interconversion of pyruvate and lactate with concomitant interconversion of NADH and $NAD^{+.}$ It is present at high levels in *Plasmodium falciparum* and *P. vivax* and is essential for energy production in parasite as they possess deficient Kreb's cycle (Wiwanitkit V., 2007 & Wu Y.S., Lei L.M. and Li M., 2005 & Dunn C. R. *et al*., 1996). It is previously noted that *plasmodial* LDH has quite different structural and kinetic properties making it different from mammalian and bacterial LDH (Dunn C. R. *et al*., 1996). So if it is not found mutated by any drug, and being highly essential for parasite, can be used as drug target. To check this possibility it could be primarily tested by dry lab methodology against some effective components of plants like mamejavo and tulsi.

HYPOTHESIS:

"Assessment of drug efficacy against *Plasmodium falciparum* and *Plasmodium vivax* in malaria, their genetic study and *in silico* study to identify new possible drug targets from herbal components."

OBJECTIVES:

- To check efficacy of Rapid Diagnostic Tests (RDTs).
- To determine level of drug resistance *in vivo*.
- *In silico* study on effect of drug on *Plasmodium* Lactate Dehydrogenase enzyme.
- *In silico* study of antimalarial property of tulsi and mamejavo.

METHODOLOGY:

- All the samples were collected from various clinical laboratories and hospitals of Valsad from June-2010 to July-2012.
- The samples were analyzed by microscopy as well as Rapid Diagnostic Test (RDT).
- Smears from the blood samples were prepared and stained by Field's stain and observed under the microscope. Parasites were counted against WBC as parasites/ µl (WHO, 2005).
- RDT SD Bioline was obtained from SD Biostandard Diagnostic Ltd. RDTs were charged with 5 µl blood. Positive results appeared as two (*Plasmodium vivax*) or three (*Plasmodium falciparum*) rose pink colour bands (WHO, 1995 & Warhurst D. C. and Williams J. E., 1996).
- Simultaneously blood samples were also collected on filter paper and dried and kept at -20°C until further processing (Sisowath C., 2009).
- Samples from malaria positive patients were also collected after 48 hrs of treatment and checked by both microscopy and RDT (WHO, 1973).
- Two *Plasmodium vivax* and two *Plasmodium falciparum* samples having greater than 25% parasitemia were selected for further molecular analysis.
- The samples were sent to The DNA laboratory for e-PCR and sequence analysis (Berwal R. *et al*., 2006).
- The sequences obtained from the DNA laboratory were further analyzed with bioinformatics software NCBI blast for getting pair wise sequence alignment (www.ncbi.nlm.nih.gov/).
- Sequences showing high level of similarity with query sequence were retrieved from the data base of NCBI. These sequences were also aligned by EMBL tool Clustal W to get multiple sequence alignment and Phylogenetic tree (www.ebi.ac.uk/embl/ & http://www.ebi.ac.uk/Tools/msa/clustalw2/).
- The structures of *Plasmodium* and human lactate dehydrogenase were retrieved from the protein data base PDB (www.rcsb.org/).
- Amino acid sequences of dihydroxypteorate synthetase were retrieved from the data base NCBI and were modelled for getting 3D protein structure with the help of Swiss model (www.swissmodel.expasy.org/).
- The data base NCBI Pub Chem and Zinc were used for data mining of structures of chemical components of *Ocimum sanctum* and *Enicostema littorale*.
- The 3D protein structures from PDB and Swissmodel were docked with Swissdock/Arguslab with different chemical components (www.swissdock.ch/ & www.arguslab.com).

RESULTS AND DISCUSSION:

Various results obtained are as below:

TABLE-1 : Microscopic diagnosis from blood

Sr. No.	*Plasmodium* species	No. of Cases
1.	*Plasmodium falciparum*	175
2.	*Plasmodium vivax*	316
3.	*Plasmodium falciparum* & *Plasmodium vivax*	07
Total		**498**

TABLE-2 : Rapid diagnostic test by chromatographic technique

Sr. No.	Organism	Negative	Positive	Total
1.	*Plasmodium falciparum*	00	182	182
2.	*Plasmodium vivax*	11	305	316
Total		**11**	**487**	**498**

TABLE-3 : Demographic study of *plasmodium* infected patients (Age wise and gender wise)

Sr. No.	Age Group	Sex		Total
		Male	Female	
1.	≤ 10 yrs	06	11	17
2.	10-30 yrs	104	68	172
3.	30-50 yrs	136	52	188
4.	50-70 yrs	75	24	99
5.	>70 yrs	14	08	22
Total		**335**	**163**	**498**

TABLE-4 Density of parasites by direct microscopy

Sr. No.	Density	Organism			Total
		Plasmodium falciparum* & *P. vivax	***Plasmodium falciparum***	***Plasmodium vivax***	
1.	+	00	07	37	44
2.	++	00	10	58	68
3.	+++	01	51	130	182
4.	++++	06	107	91	204
Total		**07**	**175**	**316**	**498**

TABLE-5 : Parasites indices of *plasmodium* infected patients

Sr. No.	Parasites/µl	*Plasmodium falciparum*	*Plasmodium vivax*	*P. falciparum* & *P. vivax*	Total
1.	1 -100	00	10	00	10
2.	101 -500	07	40	00	47
3.	501 -5,000	55	164	01	220
4.	5,001 -25,000	54	78	05	137
5.	25,001 – 50,000	27	15	01	43
6.	50,001 – 1,00,000	19	09	00	28
7.	>1,00,000 (TNTC)	13	00	00	13
Total		**175**	**316**	**07**	**498**

TNTC: To Numerous To Count

TABLE-6 : Parasitemia after 48 hours of infection

Sr. No.	Criteria	
1.	00% Parasitemia found	422
2.	<25% Parasitemia found	67
3.	>25% Parasitemia found	09
Total		**498**

TABLE-7 : Age wise parasitemia indices

Sr. No.	Age (Years)	Gender	Microscopic Diagnosis	Parasite Density (Before treatment)	Parasite Density (After treatment-48 hrs)	Parasites/µl (Before treatment)	Parasites/µl (After treatment-48 hrs)	Drug used for treatment
1.	5	Female	*Plasmodium falciparum*	+++	+++	7151	3571	Artemether+ Lumefantrine
2	48	Male	*Plasmodium falciparum*	++++	++++	230303	64000	Arteether
3.	2	Male	*Plasmodium falciparum*	++++	++++	19607	13334	Artemether+ Lumefantrine
4.	54	Male	*Plasmodium falciparum*	++	+	663	242	Arteether
5.	25	Male	*Plasmodium vivax*	++	+	1450	417	Chloroquine+Primaquine
6.	44	Male	*Plasmodium vivax*	+++	++	1714	647	Chloroquine+Primaquine
7.	19	Male	*Plasmodium vivax*	++++	+++	4075	1904	Chloroquine+Primaquine
8.	64	Male	*Plasmodium vivax*	+++	+++	2807	2060	Chloroquine+Primaquine
9.	63	Male	*Plasmodium vivax*	++++	++	3921	1377	Chloroquine+Primaquine

TABLE-8 : Sequences of parasite lactate dehydrogenase obtained from samples with >25% parasitemia

Sr. No.	Sample ID	Organism	Primer	Sequence obtained
1.	APV 2	*Plasmodium vivax*	5' TAACAAGGACTTCCAAGC 3'	atgacgccga aacccaaaat tgtgctcgtc gggtcgggca tgatcggagg cgtgagggcc acgctgattg gcagaagaa cctgtgggac gtagtgatgt ttgacgtagt gaaaaacatg ccccaaggaa aggcactaga
2.	APV 4	*Plasmodium vivax*	5' TAACAAGGACTTCCAAGC 3'	atgacgccga aacccaaaat tgtgctcgtc gggtcgggca tgatcggagg cgtgagggcc acgctgattg gcagaagaa cctgtgggac gtagtgatgt ttgacgtagt gaaaaacatg ccccaaggaa aggcactaga
3.	BPF 3	*Plasmodium falciparum*	5' GCTCAAAGATACAAATATAAGC 3 '	atggcaccaa aagcgaaaat cgttttagtt ggctcaggta tgattggagg agtaatggct accttaattg tcagaaaaa tttaggagat gtagttttgt tcgatattgt aaagaacatg ccacatggaa aagctttaga
4.	APF 2	*Plasmodium falciparum*	5' GCTCAAAGATACAAATATAAGC 3'	atggcaccaa aagcgaaaat cgttttagtt ggctcaggta tgattggagg agtaatggct accttaattg tcagaaaaa tttaggagat gtagttttgt tcgatattgt aaagaacatg ccacatggaa aagctttaga

The pair wise sequence alignment with NCBI BLAST and multiple sequence alignment with Clustal W of above *Plasmodium falciparum* and *Plasmodium vivax* of LDH sequences shows complete similarity with *Plasmodium falciparum* 3D7 strain and *Plasmodium vivax* ori I strains respectively. The *P. falciparum* and *P. vivax* sample strains are phyllogenetically found closely related with *Plasmodium falciparum* isolate mzr I LDH and *Plasmodium vivax* isolate ori I LDH respectively. Moreover highest e-values given by Argus lab software for protein pLDH with PDB ID 1CET, 2AA3 and 1T2C are observed -9.52 (carvacrol), -10.2 (luteolin) and -8.34 (rosmarinic acid) Kcal/mol respectively.

CONCLUSION:

RDTs can be definitely used for malaria diagnosis instead of conventional microscopy, as it is faster, easier, reliable and even cheaper. Out of 498 samples, 9 (1.8%) samples were suspected to have antimalarial drug resistance. Present study shows no mutational changes in the pLDH even in drug resistant samples, suggesting it as a good target for new antimalarial drug discovery. Especially plants components of mamejavo and tulsi like carvacrol, luteolin and rosmarinic acid- may prove good antimalarial agents.

REFERENCES:

- Ahmed S. E., Mohamed T. E. H., Al Sharieef G. M. G., Arsod M. M. F. and Malik E. F. M., The use of rapid diagnostic test (Parachek-Pf) in an area of low malaria transmission, Khartoum, Sudan, *Sudanese Journal of Public Health*, **2 (2)**: 89-94 **(2007)**.
- Assefa A., Kassa M., Tadese G., Mohamed H., Animut A. and Mengesha T., Therapeutic efficacy of coartem against *Plasmodium falciparum* in KERSA, South West Ethiopia, *Bio. Med. Central.*, **3 (1)**: 1-9 **(2010)**.
- Basco L. K., Marquet F., Makler M. M. and Bras J. L., *Plasmodium falciparum* and *Plasmodium vivax*, Lactate Dehydrogenaste Activity and its Application for *in vitro* drug Susceptibility Assay, *Experimental Parasitology*, **80**: 260-271 **(1995)**.
- Berwal R., Gopalan N., Chandel K., Prakash S. and Sekhar K., Amplification of LDH gene from Indian strains of *Plasmodiun vivax*, *J. Vect. Dorne. Dis.*, **43**: 109-114 **(2006)**.
- Chadee D. D., Maitre A. L. and Tillukdharry C. C., An outbreak of *Plasmodium vivax* malaria in Trinidad, *W. I. Ann. Trop. Med. Parasitol.,* **45**: 583-590 **(1992)**.
- Chaikuad A., Fairweather V., Conners R., Joseph-Horne T., Turgut-Balik D. and Brady R. L., Structure of lactate dehydrogenase from *Plasmodium vivax*: complexes with NADH and APADH, *Biochemistry*, **44**:16221–16228 **(2005)**.
- Das S. K. and Vasudevan D. M., Tulsi: The Indian holy power plant, *s*, **5(4)**, 279-283 **(2006)**
- Dunn C. R., Banfield M. J., Barker J. J., Higham C. W., MoretonK. M., Turgut-Balik D., Brady R. L. and Holbrook J. J., The structure of lactate dehydrogenase from *Plasmodium falciparum* reveals a new target for anti-malarial design, *Nature Struct. Biol.*; **3**: 912–915 **(1996)**.
- Guidelines for treatment and diagnosis of malaria in India **(2011)**.
- Hoffman S. L., Diagnosis, treatment and prevention of malaria, *Medical clinics of north America*, **76, No. 6**: 1327-1355 **(1992)**.
- Howard R. J. *et al.*, Secretion of malaria Histidine-rich protein from *Plasmodium falciparum*- infected erythrocytes, *J. Cell. Biol.*, **103**: 1269-1277 **(1986)**.

- Iqbal J., Sher A., Hira R. P. and Al-owaish R., Comparison of the OptiMAL Test with PCR for Diagnosis of Malaria in Immigrants, *Journal of Clinical Microbiology*, **37, No. 11**: 3644-3646 **(1999)**.
- Kalra B. S., Chawla S., Gupta P. and Valecha N., Screening of Antimalarial Drugs : An Overview, *Indian J. Pharmacol.,* **38 (Issue 1)**: 5-12 **(2006)**.
- Katewa S. S. and Arora A., *Indian Drugs,* **38(1)**, 6 **(2001)**.
- Kumar A., Valecha N., Jain T. and Dash A. P., Burden of Malaria in India: Retrospective and Prospective View, *Am. J. Trop. Med. Hyg.*, **77 (Suppl. 6)**: 69–78 **(2007)**.
- Kumar K. R. and Sudarshan K. S., Clinical evaluation of a rapid diagnostic kit (Parachek-Pf) for diagnosis of *Plasmodium falciparum* in Karnataka state of India, *Indian J. Pred. Soc. Med.*, **35 (1, 2)**: 10-14 **(2004)**.
- Lim H. S. and Kim H. S., Evaluation of diagnostic methods of re-emerging malaria in Korean patients, *Yonsei Medical Journal*, **42 (1)**: 84-90 **(2001)**.
- Makler M. T. and Hinrichs D. J., Measurement of the lactate dehydrogenase activity of *Plasmodium falciparum* as an assessment of parasitemia, *Am. J. Trop. Med. Hyg.*, **48**: 205-210 **(1993)**.
- Makler M. T., Palmer C. J. and Ager A. L., A review of practical techniques for the diagnosis of malaria, *Ann. Trop. Med. Parasitol.*, **92**: 419-33 **(1998)**.
- Noedl H. *et al.*, Histidine-Rich Protein II, A Novel Approach to Malaria Drug Sensitivity Testing, *Antimicrobial Agents and Chemotherapy*, **46(6)**: 1658-1664 **(2002)**.
- Nussenzweig R. S. and Zavala F., A malaria vaccine based on a sporozoite antigen, *N. Engl. J. Med*., **336**: 128-130 **(1997)**.
- Penna-Coutinho J., Cortopassi W. A., Oliveira A. A., Costa França T. C. and Krettli A. U., Antimalarial Activity of Potential Inhibitors of *Plasmodium falciparum* Lactate Dehydrogenase Enzyme Selected by Docking Studies, *PLoS ONE (www.plosone.org)*, **6 (Issue 7)** : e21237 **(2011)**.
- Pinchart M. P. B., Pinel C., Croisonnier A., Brion J. P., Faure O., Ponard D. and Thomas P. A., Diagnosis of Malaria in non-endemic countries by the Parasight-F test, *Am. J. Trop. Med. Hyg.*, **63 (3, 4)**: 150-152 **(2000)**.
- Prakash N., Patel S., Faldu N., Ranjan R. and Sudheer D. V. N., Molecular docking studies of antimalarial drugs for malaria*, J. Comput. Sci. Syst. Biol.*, **3 (3)**: 70-73 **(2010)**.
- Rodulfo H., Donato M. D., Mora R., Gonzalez L. and Contreras C. E., Comparison of the diagnosis of malaria by microscopy, immunochromatography and PCR in endemic area of VENEZUELA, *Brazilian Journal of Medical and Biological Research*, **40**: 535-543 **(2007)**.
- Rougemont M., Saanen M. V. and Sahli R. *et al.*, Detection of four *Plasmodium* species in blood from humans by 18S rRNA gene subunit-based and species-specific real-time PCR assays, *J. Clin. Microbiol.*, **42**: 5636-43 **(2004)**.
- Sharma P.,Re-emergence of malaria in India. *Indian J. Med. Parasitol.*, **103:** 26-45 **(1996)**.
- Singh N. and Misra K., Computational screening of molecular targets in *Plasmodium* for novel non-resistant anti-malarial drugs, Bioinformation, **3 (6)**: 255-262 **(2009)**.
- Singh N., Valecha M. and Sharma V. P., Malaria diagnosis by field workers using an immune chromatographic test, *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **91(4)**: 396-397 **(1997)**.
- Sisowath C., The molecular basis of *Plasmodium falciparum* resistance to the antimalarial Lumefantrine, Karolinska Institetet, Stockholm **(2009)**.
- Srivastava H. C. and Yadav R. S., Malaria outbreaks in tribal area of Gujarat state, India **(2000)**.
- Stepniewska K. *et al.*, *In vivo* assessment of drug efficacy against *Plasmodium falciparum* malaria: Duration of follow-up, *Antimicrobial agents and chemotherapy*, **48 (11)**: 4271-4280 **(2004)**.
- Warhurst D. C. and Williams J. E., Laboratory diagnosis of malaria, ACP broadsheet No. 148, *J. Clin. Pathol*, **49**, 533-538 (**1996)**.

- White N. J., The assessment of antimalarial drug efficacy, *Trends Parasitol.,* **18**: 458– 464 **(2002)**.
- Wiwanitkit B., *Plasmodium* and host LDH molecular function and biological pathways: Implication for antimalarial drug discovery, Chulalongkorn University, Thailand **(2007)**.
- Wu Y.S., Lei L.M. and Li M., Evaluation of a parasite lactate dehydrogenase-based colloid gold-immunochromatography assay for diagnosis of *Plasmodium falciparum*, *Di Yi Jun Yi Da XueXue Bao*, **25**: 761–765 **(2005)**.
- World Health Organization, Chemotherapy of Malaria and Resistance to Antimalarials, Report of a WHO scientific group, *WHO Technical Report Series*, No. 529, Geneva, WHO **(1973)**.
- World Health Organization, Management of uncomplicated malaria and the use of antimalarial drugs for the protection of travellers, Information consultation report, Geneva, WHO/MAL, 1075-98, (**1995)**.
- World Health Organization, *Manual of basic techniques for a health laboratory*, 2nd edition, (**2005)**.

❖ **Web References**

- www.arguslab.com
- www.ebi.ac.uk/embl/
- http://www.ebi.ac.uk/Tools/msa/clustalw2/
- http://www.flutrackers.com/forum/showthread.php?t=170884.
- http://www.malariasite.com
- http://www.mrcindia.org/Guidelines%20for%20Diagnosis2011.pdf
- www.ncbi.nlm.nih.gov/
- www.rcsb.org/
- www.swissdock.ch/
- www.swissmodel.expasy.org/

Student: ***Guide:***

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